
# Study on ochratoxin A contamination of coffee batches in the Kenyan context, in relation to cultivation methods and post harvest processing treatments

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# Study on ochratoxin A contamination of coffee batches in the Kenyan context, in relation to cultivation methods and post-harvest processing treatments

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**Abstract**

This study set out to assess the relative importance of sound beans and unsound beans in a batch of coffee with regard to ochratoxin A (OTA) contamination. In first stage, unsound beans were found to account for 95% of contamination in a batch of coffee, whatever the methods used for post-harvest processing. It was also found that beans displaying traces of attacks by *Colletotrichum kahawae* were the greatest contributors to OTA contamination. In a second stage, the study compared the contamination of sound beans with that of beans attacked by *Colletotrichum kahawae.* On average, beans attacked by *Colletotrichum kahawae* had a statistically higher OTA content than sound beans (18.0 $\mu g.kg^{-1}$ as opposed to 1.2 $\mu g.kg^{-1}$). In addition, the average OTA content in unsound beans varied depending on growing conditions

**Keywords:** *Coffea arabica,* unsound beans, coffee berry disease, ochratoxin A

## Introduction

OTA existence in coffee beans can be considered in relation to harvesting conditions (Moreas et al. 2003), post-harvest processing conditions (Bucheli et *al*.2000; Joosten et *al*. 2001; Suarez-Quiroz et *al*. 2004; Romani et *al*. 2004), especially during dry processing (Moreas et *al*. 2003; Suarez-Quiroz et *al*. 2004; Bucheli et *al*. 1998; Urbano et *al*. 2001*),* and storage and transportation conditions (Bucheli et *al*. 1998). In the literature cited, OTA quantification was generally carried out on unsorted coffees (Urbano et *al*. 2001) sampled after processing. This coffee always contains varying proportions of unsound beans depending on the quality of cultural and technological production factors. On the other hand, no OTA quantification is done on samples taken before processing assuming that OTA contamination occurs only during the processing. Now, OTA has been detected in beans extracted from fresh cherries with, sometimes, high levels of OTA (> 5 $\mu g.kg^{-1}$). This observation (not published) lets suppose that OTA contamination occurs in orchard and might affect certain beans. The hypothesis which has been formulated is that defects might significantly contribute to OTA contamination in a batch of unsorted beans. To verify it, a study has been carried out in Kenya where there is a perfect traceability of coffee and where two processing methods are commonly used.

Coffee production in Kenya is shared between producers grouped in farmer cooperative societies (FCS) and estates (E). FCS farmers practise extensive cultivation of a few acres of coffee trees and use very few or no inputs. Conversely, estates practise intensive coffee cultivation, where estates are kept in good phytosanitary condition by regular applications of fungicides and insecticides. Kenyan producers retain ownership of their coffee up to its sale to exporters or roasters. The price paid for coffee depends on its quality when it goes up for auction. That therefore calls for perfect traceability of the coffee from the production unit to the warehouses in which the coffee entering the market is stored.

The production unit is defined as being the structure comprising the plantation from which the cherries are harvested, and the washing stations where the cherries undergo their first post-harvest treatment. In the case of the FCSs, the production unit involves several hundred to several thousand farmers, who deliver their production to a community washing station. For the estates, the plantation and washing station belong to the same person.

A production unit produces coffee in the form of dry cherries (mbuni) and parchment coffee at a moisture content of 10-11%. The coffee is referenced then and sent to hulling and

grading units where it undergoes a second process to prepare commercial batches for export. Whilst the farming systems in Kenya might be highly contrasting, coffee cherries undergo the same post-harvest processing treatments (figure 1), during which perfectly codified procedures are applied identically, irrespective of the production unit or hulling factory.

## Material and methods

The study was conducted in two stages :

- firstly, distribution by bean type (sound and unsound beans) and their contamination by OTA were assessed on coffee samples (S1a and S1b) obtained directly from production units, after initial post-harvest processing (figure 1).
- secondly, coffee samples (S2) were taken from the hulling factories (figure 1), in order to assess OTA contents for different types of defects encountered, and to study the contamination of a particular defect chosen in line with the results of the previous study. The type of defect selected for the second phase of the study contributed most to the overall contamination of a coffee batch.

### Coffee samples

- ***Coffee from production units***

After picking, cherries were brought to the washing station (figure 1) where they were first sorted by hand. Sound and slightly damaged cherries were pulped and the mucilage removed by fermentation for 12 hours (wet processing). They were then washed and sun dried. That gave parchment coffee. During washing, heavy parchment coffee (P1) was separated by densimetric sorting from light parchment coffee (PL). Unripe, overripe and cherries severely damaged by insects or diseases were separated off and spread on dryers (dry processing). After drying, dry cherries, or mbuni, were obtained. Both processing methods existed side by side in the same production unit and application of either method depended on initial cherry quality.

130 kg of light parchment coffee (S1a) and 200 kg of mbuni (S1b) were collected from two different cooperatives, i.e. the equivalent of 100 kg of beans (or green coffee) per cooperative. Light parchment coffee and mbuni were chosen to increase the probability of obtaining a sufficient quantity of defective beans. After hulling, the coffee was sorted by hand and classed into different bean categories. For each bean category, a 100 to 150-g sample was

taken for OTA quantification. The samples were then placed in paper bags and sent to the laboratory.

- ***Coffee from hulling and grading factories***

Coffee from the washing stations was first hulled and the beans were separated from the shell or parchment. They were then passed on to densimetric sorting tables, where they were classed according to their density and size (grading), then sorted by colorimetry to separate unsound beans (stained beans, broken beans, abnormally coloured beans) from sound beans. As the aim was to add as much value as possible to each producer's coffee, each batch of heavy parchment, light parchment and mbuni from the same production unit was processed separately. Each initial batch of parchment coffee thus gave different categories of green coffee. The upper categories corresponding to commercial grades AA, AB, C and PB contained large, sound beans without defects, and the intermediate categories (commercial grades C, T and TT) contained smaller beans and a small percentage of defects (<2-3%). However, the lower categories (grade SB) comprised a mixture of sound beans and varying proportions of unsound beans, apart from those strictly refused by roasters. Coffee produced from mbuni was roughly sorted to remove undesirable black beans and stinkers.

In order to collect enough unsound beans, samples (S2) (figure 1) were taken from coffees in the lower categories (SB) derived from light parchment coffee (S2a) and mbuni (S2b). For each sample, the number of the processed batch was recorded in order to know its origin: type of production unit (FCS or estate), post-harvest processing method. A total of 75 1- to 2-kg bean samples, were taken at random during processing at the hulling and sorting factories. The beans were sorted and classed into sound beans and seven different types of defects, ultimately giving 229 sub-samples on which OTA contents were determined.

**Analyses of OTA in beans**

Coffee samples were frozen at –80°C then ground to pass through a 0.5 mm sieve and analysed for OTA (Nakajima et *al*. 1997). The samples were extracted for 30 min with a solution of methanol/3% sodium bicarbonate (50:50); the extracts were filtered and diluted with phosphate-buffered saline and applied to an immunoaffinity column (Ochratest®, Rhône Diagnostics, Scotland). OTA was eluted with 3 ml HPLC grade methanol. The eluate was evaporated to dryness under a stream of nitrogen at 70°C and the residue was redissolved in

1 ml of HPLC mobile phase then quantified by HPLC (Shimadzu LC-10ADVP, Japan, with a fluorescence detector). The mobile phase consisted of distilled water/acetonitrile/glacial acetic acid (51:48:1). The flow rate was 1 ml/min. OTA was detected at an excitation wavelength of 333 nm and an emission wavelength of 460 nm, and a retention time of 13.3 – 13.5 min. Standard OTA curves were established with an ochratoxin standard (1000 $ng.ml^{-1}$; (ref PD 226 R. Biopharm Rhône Ltd, Scotland); the detection limit was 0.03 $ng.ml^{-1}$.

**Statistical analyses**

Analyses of variance were performed after $x \rightarrow \ln(x)$ log transformation, where x was the OTA content of the sample expressed in $\mu g.kg^{-1}$. For non-quantifiable contents (traces), OTA content was fixed arbitrarily at the detection limit (0.03 $\mu g.kg^{-1}$). The software used was R (The R Manuals edited by the R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2006, ISBN3-900051-07-0, www.R-project.org)

## Results and discussion

### Coffee from production units

- ***Types and distribution of defects:***

At the end of manual sorting, seven categories of beans were identified, for both parchment coffee (PL) and mbuni coffee (table 1). Sound beans accounted for 75.3% of total bean weight in parchment coffee and 64.9% of total bean weight in mbuni. That result tallied with the preliminary sorting of cherries on arrival at the washing station.

Of the six categories of defects, beans attacked by *Colletotrichum kahawae* (diseased beans) and insect damaged beans were defects of agronomic origin. Hulled ears were due to polyembryony. Foxy beans and stinkers came from a lack of care during post-harvest processing and were defects systematically eliminated from commercial batches. As sorting criteria were mostly based on bean colour, it was often impossible to distinguish between foxy beans and diseased beans with certainty, and the proportion of these latter defects was probably underestimated. The origin of black beans was poorly determined and may have been agronomic or technological.

Defects of agronomic origin were the most frequent, with a strong predominance of diseased beans. Those beans, which were characterized by brownish patches on the surface of the beans, came from cherries subjected to late coffee berry disease (CBD) attacks. In fact, beyond the 26th or 27th week after flowering (i.e. around 1.5 months before harvest),

fungicide treatments were halted because the disease does not lead to harvest losses but to external cherry damage. The larger proportion of diseased beans in mbuni (59% as opposed to 46% for parchment coffee) confirmed the merits of sorting before post-harvest processing.

- ***OTA contamination:***

All in all, the sound beans had a very low OTA content (0.2 $\mu g.kg^{-1}$ for parchment coffee and 0.4 $\mu g.kg^{-1}$ for mbuni), while the weighted contamination for defects reached 8.8 and 10.3 $\mu g.kg^{-1}$ respectively (table 1). Under our study conditions, the existence of defects could account for more than 98% of the contamination in a batch of coffee. Diseased beans appeared to be a major contributor to contamination in parchment coffee, and probably in mbuni given the uncertainty surrounding foxy beans. Defects of technological origin were weakly contaminated and there did not seem to be any post-harvest process effect on contamination levels, thereby confirming the hypothesis put forward by Suarez-Quiroz et *al.* 2004. Lastly, eliminating the defect contributing most to average batch contamination would appear to reduce contamination to 1.0 $\mu g.kg^{-1}$ for parchment coffee (PL) and 6.4 $\mu g.kg^{-1}$ for mbuni, as opposed to 9.2 $\mu g.kg^{-1}$ and 12.0 $\mu g.kg^{-1}$.

**Coffees from hulling and grading factories**

The average OTA contents of sound beans and the seven types of defects were grouped into five categories (table 2) and confirmed earlier observations. Apart from foxy beans, which can be confused with beans damaged by CBD, defects of agronomic origin were the most contaminated. Statistical analyses were performed to compare the OTA contents of sound beans and diseased beans, based on the processing method applied to cherries (dry or wet), the growing system (intensive, extensive). Remember that beans were sorted by hand before post-harvest processing and the process applied depended solely on the initial condition of the cherries, which was judged visually.

- ***Wet processing versus dry processing:***

Whatever the post-harvest process the farming system, diseased beans were statistically more contaminated than sound beans (18.0 and 1.2 $\mu g.kg^{-1}$ respectively).

Given that each batch of coffee delivered by the production units to the hulling/grading factories was perfectly identified and processed separately, it was possible to ascertain the background of each sample. It was therefore possible to compare the two types of beans and the post-harvest processing methods applied (table 3). Diseased beans from mbuni or parchment coffee had OTA contents of 29.7 and 10.6 $\mu g.kg^{-1}$ respectively and there

was a significant difference between the dry method and the wet method. That same statistical difference was also found for sound beans (1.9 and 1.0 µg.kg$^{-1}$ respectively). Those differences could be explained by the fact that the most contaminated beans came from cherries processed by the dry method (mbuni) where less care is applied along drying course, thereby confirming the effect of the post-harvest process (Moreas et *al*. 2003; Bucheli et *al*.2000; Joosten et *al*. 2001). However, as the most severely damaged beans were processed into mbuni, it was logical to find greater contamination in that type of coffee.

- ***Intensive estate system versus extensive FCS system***

CBD symptoms on cherries indicate that fruit integrity has not been preserved, thereby facilitating the installation of saprophytic fungi. In brief, CBD development in the absence of any control by fungicides leads to substantial fruit-fall up to the 24th-26th week after flowering. Thereafter, attacks are considered as late and, in principle, they do not cause harvest losses but can leave traces on beans. Spraying contact fungicides monthly up to the 6th or 7th month after flowering effectively controls the disease (Masaba et *al.* 1992; Bieysse et *al*. 2002). It was mentioned earlier that the degree of intensification in coffee plantations was relatively low among smallholders. In particular, input use was very low or nonexistent for a great majority of them. Consequently, berries were not protected or inadequately protected from CBD.

The statistical analyses shown in table 4 reveal a significant difference between OTA contents for diseased beans (29.3 µg.kg$^{-1}$) and sound beans (1.2 µg.kg$^{-1}$) in the case of the FCSs and no difference in the case of the estates (4.3 µg.kg$^{-1}$ as opposed to 1.2 µg.kg$^{-1}$). OTA contents in diseased beans were also statistically different between the FCSs and the estates, whilst sound beans have similar OTA contents. It therefore seems that preventive measures against CBD also have a favourable effect as regards OTA contamination. During this study, it was not possible to elucidate how the fungicides used against CBD acted in the reduction of OTA contamination. It may have involved late contamination by toxigenic moulds leading to relatively low OTA production, or direct action against the moulds, with the fungicides used slowing down their development. It should be noted that the producers used both contact fungicides (copper-based or synthetic) and systemic fungicides.

A final series of statistical analyses (table 4) was carried out to check the impact of sorting before post-harvest processing. For the FCSs, diseased beans and sound beans derived from mbuni were always more contaminated than those derived from parchment coffee (PL). For the estates, no significant difference was noted, be it for sound beans or diseased beans.

This result confirmed the merits of sorting prior to post-harvest processing, especially in the case of the FCSs. On the other hand, it is likely that the processing method, dry or wet, only had a negligible influence. It would therefore seem that the degree of bean contamination at the end of post-harvest processing depended considerably on cherry contamination at the moment they were harvested.

## Conclusions

Given the very good traceability of Kenyan coffee, it was possible to show that bean contamination by OTA is probably highly dependent upon the quality and sanitary condition of cherries at the moment they are harvested. In particular, defects of agronomic origin could be the main contributors to OTA contamination. In Kenya, CBD-damaged beans are without any doubt the cause of high OTA contents that can be found in inferior quality batches. Insect-damaged beans (CBB, *Antestia,* fruit flies) could definitely play a role in coffee contamination (Vega et *al*. 1999) when commercial batches are only roughly sorted. The results presented in this study need to be compared to those obtained on grapevines and wine (Rousseau 2003). Indeed, it has been shown that grapes damaged by fruit flies are more contaminated than sound grapes. The results of this study could provide the basis for field trials, more particularly to assess the impact of effective phytosanitary protection in terms of food safety.

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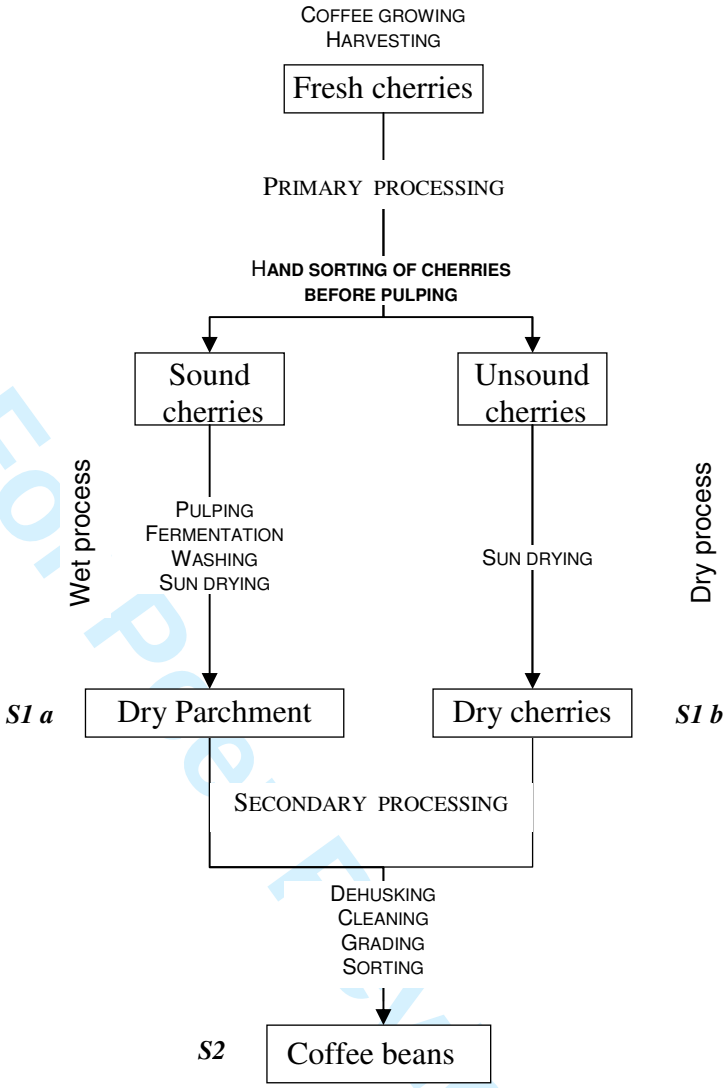


**Figure 1:** Coffee flow and processing steps from fresh cherries to coffee beans in Kenya
***S1 a, S1 b et S2*** : sampling steps

**Table 1 :** Percentage of different types of beans after hand sorting, OTA contents in $\mu g.kg^{-1}$ and weighted contamination of beans from PL and Mbuni

| Types of coffee | Parchment light (PL) | | | | Mbuni | | | |
|---|---|---|---|---|---|---|---|---|
| Types of beans | Weight (%) | OTA ( $\mu g.kg^{-1}$ ) | | % cont. | Weight (%) | OTA ( $\mu g.kg^{-1}$ ) | | % cont. |
| | | a | b | | | a | b | |
| Black beans | 1,8 | 2,4 | 1,85 | 0,37 | 7,6 | 1,6 | 1,63 | 1,04 |
| Diseased beans | 11,3 | 71,3 | 73,29 | 90,41 | 20,6 | 37,5 | 33,65 | 57,65 |
| Foxy beans | 0,6 | 5 | 2,79 | 0,19 | 1,4 | 291,6 | 315,19 | 37,89 |
| Hulled ears | 10,3 | 0,4 | 0,39 | 0,43 | 3,9 | 0,9 | 0,58 | 0,19 |
| Insect damaged beans | 0,5 | 46,2 | 134,91 | 7,52 | 1,2 | 9 | 9,17 | 0,89 |
| Stinkers | 0,1 | 3,1 | 3,73 | 0,05 | 0,3 | 2,3 | 1,43 | 0,04 |
| Defects | 24,7 | na | 8,79 | 98,97 | 35,1 | na | 10,31 | 97,70 |
| Sound beans | 75,3 | 0,2 | 0,13 | 1,03 | 64,9 | 0,4 | 0,43 | 2,30 |
| Total | 100,0 | na | 9,2 | - | 100,0 | na | 12,0 | - |

a Means of 2 replicates x 2 batches of 100 kg of beans
b Weighted means
% cont. : contribution (%) of each defect in total contamination

**Table 2:** Means by class of OTA contamination for each type of beans

| Types of beans (total of samples) | | Means of OTA contents (µg.kg$^{-1}$) by class | | | | |
|---|---|---|---|---|---|---|
| | | traces | <1 | 1-9,9 | 10-99,9 | >=100 |
| Sound beans 75 | number | 6 | 47 | 22 | - | - |
| | mean | na | 0.3 | 3.6 | - | - |
| | s.d. | na | 0.2 | 2.4 | - | - |
| Diseased beans 62 | number | 8 | 19 | 21 | 11 | 3 |
| | mean | na | 0.4 | 3.7 | 54.7 | 142.9 |
| | s.d. | na | 0.3 | 2.1 | 26.4 | 11.6 |
| Black beans 32 | number | 1 | 11 | 16 | 2 | 2 |
| | mean | na | 0.4 | 2.6 | 37.2 | 118.1 |
| | s.d. | na | 0.3 | 1.2 | 35.2 | 18.9 |
| Insect damaged beans 16 | number | 3 | 4 | 4 | 4 | 1 |
| | mean | na | 0.5 | 3.6 | 29.3 | 499.3 |
| | s.d. | na | 0.3 | 1.7 | 30.9 | na |
| Foxy beans 16 | number | 2 | 2 | 4 | 3 | 4 |
| | mean | na | 0.2 | 5.3 | 29.7 | 389.8 |
| | s.d. | na | 0 | 1.7 | 18.9 | 248.4 |
| Stinkers 10 | number | - | 1 | 8 | 1 | - |
| | mean | - | 0.1 | 3.1 | 32.1 | - |
| | s.d. | - | na | 1.3 | na | - |
| Hulled ears 14 | number | 1 | 10 | 2 | 1 | - |
| | mean | na | 0.4 | 1.7 | 29.6 | - |
| | s.d. | na | 0.2 | 0 | na | - |
| Others 4 | number | - | - | 3 | 1 | - |
| | mean | - | - | 2.4 | | - |
| | s.d. | - | - | 2.3 | na | - |

na : not available – s.d. : standard deviation

Table 3 : OTA contents (µg.kg$^{-1}$) in diseased and sound beans in buni and PL for both sectors of production

| Source of variation | | Mean | Statistics (p=0.95) | | Conclusions |
|---|---|---|---|---|---|
| | | | df | F | |
| All beans | Diseased | 18.0 | 1-135 | **15.277** | Diseased >Sound |
| | Sound | 1.2 | | | |
| Diseased | Buni | 29.7 | 1-60 | **10.308** | Buni > PL |
| | PL | 10.6 | | | |
| Sound | Buni | 1.9 | 1-73 | **5.381** | Buni > PL |
| | PL | 1.0 | | | |

In bold, significant difference

Table 4 : OTA contents (µg.kg$^{-1}$) in diseased and sound beans from FCS and Estates

| Source of variation | | Mean | Statistics (p =0.95) | | Conclusions |
|---|---|---|---|---|---|
| | | | df | F | |
| FCS | Diseased | 29.3 | 1-67 | **13.506** | Diseased >Sound |
| | Sound | 1.2 | | | |
| E | Diseased | 4.3 | 1-66 | 3.330 | Diseased =Sound |
| | Sound | 1.2 | | | |
| Diseased | FCS | 29.3 | 1-60 | **5.067** | FCS>E |
| | E | 4.3 | | | |
| Sound | FCS | 1.2 | 1-73 | 0.938 | FCS = E |
| | E | 1.2 | | | |
| Diseased FCS | Buni | 38.7 | 1-32 | **10.966** | Buni > PL |
| | PL | 20.0 | | | |
| Diseased Estates | Buni | 8.0 | 1-26 | 0.076 | Buni = PL |
| | PL | 3.0 | | | |
| Sound FCS | Buni | 2.0 | 1-33 | **16.307** | Buni > PL |
| | PL | 0.6 | | | |
| Sound Estates | Buni | 1.1 | 1-26 | 0.706 | Buni = PL |
| | PL | 0.5 | | | |

In bold, significant difference